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Immune Responses to Inactivated Vaccine in People Naturally Infected With Hantaviruses

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An inactivated Hantaan virus vaccine for hemorrhagic fever with renal syndrome (HFRS) was given by injection to 15 people who were naturally infected with either Hantaan or Seoul viruses. Immunofluorescent antibody (IFA), reversed passive hemagglutination inhibition (RPHI), hemagglutination inhibition (HI), and neutralization antibody (NA) assays were used to measure the antibody titers of the vaccinated people before and after three doses of vaccine. The results indicated that IFA and RPHI antibody titers were boosted significantly ($P < 0.05$) after the vaccination. Either Hantaan or Seoul virus could induce two-way cross-reactive neutralization antibody responses in humans. After HTNV vaccine immunization, the NA titers of people with natural infection increased significantly ($P < 0.05$) to both Hantaan and Seoul viruses, while the relative dominance between these two type responses was still similar to that of natural infection. It is worthwhile to studying the procedure further to inoculate two different virus vaccines for improving the cross-protective effect.

KEY WORDS: Hantaviruses, natural infection in humans, inactivated vaccine, antibody

INTRODUCTION

Hantavirus strain Z10, classified as Hantaan virus, was isolated from the blood sample of a patient with hemorrhagic fever with renal syndrome (HFRS) in a rural area of Zhejiang province, China [Zhu Zhiyong et al., 1988]. Strain Z10 with higher titer virus antigen and hemagglutinin were successfully cultured into primary cells from *Meriones unguiculatus* kidney. From the culture, an inactivated HTNV vaccine for HFRS was prepared at our laboratory [Zhu Zhiyiong et al., 1989]. The vaccine can induce effective homologous neutralizing antibody (NA) with 1:58.5 geometric mean titer (GMT) and 90% seroconversion rate in humans and but lower heterologous neutralizing effect [Zhu Zhiyong et al., 1990a]. However, the results in immunized *M. unguiculatus* indicated that the vaccine could cross-protect against both HTNV and Seoul virus (SEOV) challenges [Zhu Zhiyong et al., 1990b]. For the present paper, we studied retrospectively 15 vaccine recipients who had been naturally infected with Hantaviruses and observed immune responses to the natural infection and vaccine immunization.

MATERIALS AND METHODS

Vaccine and Recipients

An inactivated strain Z10 vaccine was prepared as described previously [Zhu Zhiyong et al., 1989]. Briefly, virus was cultured into primary cells from *M. unguiculatus* kidney and inactivated by β-propiolactone. After endorsement by the Ministry of Chinese National Health and the Chinese National Institute for the Control of Pharmaceutical and Biological Products (NICPBP), the farmers and students, aged 16–50 years, in an HFRS epidemic area of Zhejiang province, China, were injected intramuscularly with 1 ml of the vaccine. Two additional doses were given on days 7 and 28. Serum samples were collected on the day of vaccination and 28 days later. The recipients had no clinically characteristic features of HFRS in their medical histories. The NA seroconversion rate was 90% after three doses of vaccine. Fifteen subjects were detected to have had serum antibody to Hantaviruses before the vaccination and were regarded as subclinically infected.

Plaque Reduction Neutralization Test With Immunoperoxidase Staining

We developed a plaque reduction neutralization test with horseradish proxidase-conjugated rabbit IgG anti-HTNV staining. HTNV (strain 76-118) and SEOV were from NICPBP. Proxidase-conjugated rabbit IgG anti-HTNV was labeled at our laboratory [Zhu Zhiyong et al., 1990c]. Briefly, serial twofold dilutions (1:5) of the

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TABLE I. Serum HI Serotypes and Antibody Titers Before and After Vaccination

Serum No.	Natural infection					Postvaccination				
	HI serotype	NA		IFA	RPHI	HI serotype	NA		IFA	RPHI
		HTNV	SEOV				HTNV	SEOV		
1	HTNV	80	10	320	20	HTNV	10,240	40	≥5,120	160
2	HTNV	1,280	20	640	160	HTNV	5,120	80	≥2,560	640
3	HTNV	2,560	20	640	20	HTNV	2,560	40	2,560	80
4	HTNV	80	20	320	320	HTNV	5,120	20	5,120	320
5	HTNV	40	<5	320	40	HTNV	640	80	1,280	320
6	HTNV	320	40	640	80	HTNV	2,560	320	≥2,560	320
7	HTNV	640	20	1,280	160	HTNV	640	80	2,560	320
8	HTNV	640	80	1,280	80	HTNV	1,280	80	2,560	320
9	—[a]	10	10	640	20	HTNV	5,120	10	2,560	1,280
10	SEOV	40	40	320	160	SEOV	160	640	5,120	640
11	SEOV	320	320	320	80	SEOV	320	640	1,280	320
12	SEOV	40	80	80	<20	SEOV	1,280	2,560	2,560	1,280
13	SEOV	80	320	320	80	SEOV	160	640	1,280	320
14	SEOV	80	320	640	40	SEOV	320	1,280	1,280	80
15	SEOV	10	320	640	80	SEOV	640	2,560	2,560	640
GMT		126	46	463	64		1,167	184	2,444	351

[a]Could not be classified.

detected sera inactivated by heating for 30 min at 56°C were mixed with an equal volume of 76–118 and SEOV, respectively, and incubated for 1 hr at 37°C. Virus-serum mixtures (0.1 ml; 30–50 PFU) were inoculated in duplicate into confluent monolayers of Vero E6 cells grown in 24-well trays (Tianjing Methyl-methacrylatepolymer Product Factory, Tianjing, China) and adsorbed for 1.5 hr at 37°C. Inoculated monolayers were overlain with media containing argrose (Sigma Chemical Co., St. Louis, MO) and incubated for 5 days at 37°C. Then, the monolayer wells were added with the dilution of peroxidase-conjugated rabbit IgG anti-HTNV and incubated for 2 hr at 37°C. Finally, the dilution of 0.01% O-dianisidine (Sino-American Biotechnology Co., Shanghai, China) and H_2O_2 were used as the revealing reagents. Plaques were enumerated by the naked eye. NA titer was expressed as the reciprocal of the highest dilution of serum resulting in ≥50% reduction of plaque numbers compared to normal sera.

Hemagglutination Inhibition Test (HI)

The serum hemagglutination inhibition antibodies (HIA) to HINV and SEOV were detected, and the serotype was identified according to the titer difference (fourfold or more) between the two viruses [Song Gan and Hang Changshou, 1990].

Indirect Immunofluorescence Assay (IFA) and Reversed Passive Hemagglutination Inhibition Assay (RPHI)

IFA and RPHI were used for detecting antibodies to both HTNV and SEOV antigens. The positive criteria titers were ≥1:20 for IFA and ≥1:10 for RPHI [Song Gan and Hang Changshou, 1990].

RESULTS

HI Serotypes

The serotypes of 15 people who were naturally infected with Hantaviruses were identified by HI. The infections in 8 cases were caused by HTNV and in 6 cases by SEOV. One subject with titers to HTNV and SEOV of 1:160 vs. 1:80 could not be classified. After the vaccination with an inactivated HTNV strain Z10 vaccine, the serotypes in 14 of 15 cases were identical to those of natural infection (see Table I).

NA Titers

NA titers of sera from 15 cases were determined and are showed in Table I. NA titers to HTNV were higher than those to SEOV (4–128-fold) in 8 serum samples with the natural infection and lower (2–32-fold) in 4 serum samples. There was no difference between NA titers to two viruses in 3 serum samples. After delivery of the inactivated HTNV vaccine intramuscularly, NA titers to HTNV and SEOV were boosted significantly ($P < 0.05$), whereas the relative dominance between NA titers to two viruses was similar to that in natural infection. In addition, the serotypes of all samples after the vaccination in the plaque reduction neutralization test corresponded with those in HI.

IFA and RPHI Antibodies

Table I shows serum IFA and RPHI antibody titers in 15 cases before and after vaccination. Antibody titers to both HTNV and SEOV antigens were boosted significantly ($P < 0.05$) after the vaccination.

Correlations of NA With HI, IFA, and RPHI Antibodies

NA to HTNV after the vaccination was positively correlated with IFA antibody ($r = 0.59$, $P < 0.05$). There were positive correlations between NA and HIA to SEOV both in the natural infection and with vaccine immunization ($r = 0.77$ and 0.72, respectively; $P < 0.05$). The others showed no statistically significant correlation (Table II).

TABLE II. Correlations of NA With HI, IFA, and RPHI Antibodies

	HI		IFA		RPHI	
Classification	r	*P*	r	*P*	r	*P*
Prevaccination						
HTNV	0.52	>0.05	0.07	>0.05	0.07	>0.05
SEOV	0.77	<0.05	0.12	>0.05	−0.17	>0.05
Postvaccination						
HTNV	0.51	>0.05	0.59	<0.05	0.02	>0.05
SEOV	0.72	<0.05	−0.19	>0.05	0.40	>0.05

DISCUSSION

There are at least six recognized viruses in the Hantavirus genus. Hantaan and Seoul viruses are the etiologic agents causing HFRS in China. The antigenicities of the virus strains isolated from patients and animals in various epidemic areas of China are different. Some strains can induce higher titers of cross-reactive NA in rabbits [Yu Yongxin et al., 1991]. There is no information on NA responses before and after vaccine immunization in people who were naturally infected with Hantaviruses. The results described herein indicate that either live Hantaan or Seoul virus can induce both homologous and heterologous NA in humans. It is interesting that both NA titers are boosted after the immunization with an inactivated Hantaan virus vaccine, whereas the relative dominance between the two types is similar to that in natural infection. We wonder whether vaccine immunization results in the reactions of immunological memory cells that have been elicited by prototype virus or immunologically competent cell sensitivities to antigen determinants in Hantaan and Seoul viruses have become different after primary virus immunization. Recently, monovalue vaccines with effective protection from homologous viruses and without good heterologous NA response have been developed. On the other hand, a mixed bivalue vaccine generated lower NA titer to both HTNV and SEOV compared to a monovalue vaccine inducing homologous NA titer to either HTNV or SEOV. Given that the primary immune is important in determining NA reactivity, it is possible to obtain a satisfactory cross-protection by designing a program of inoculating different virus vaccines.

Three people naturally infected with Hantaviruses had the same NA titers to HTNV and SEOV. After the vaccination with an inactivated HTNV vaccine, one of these people had a significantly increased antibody response to HTNV. The other two generated a comparatively higher response to SEOV. Because of individual differences, it is likely that the antibody titers in these three people had become the same with the decline of the antibody level. One case with a higher NA titer to HTNV after the vaccination was supposed to have had a HTNV infection before. The primary infection of the other cases with higher NA titer to SEOV after the vaccination might have been caused by SEOV. The serotypes of all samples after vaccination in the plaque reduction neutralization test corresponded to those in HI, which confirmed that the plaque reduction neutralization test with horseradish proxidase-conjugated rabbit IgG anti-HTNV staining was reliable.

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